

**CLAIMS**

1. A monoclonal antibody that specifically binds to a human VEGF with dissociation constant  $K_d$  equal to or lower than 0.2 nM.

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2. The monoclonal antibody of claim 1, wherein the dissociation constant  $K_d$  is equal to or lower than 0.1 nM.

3. The monoclonal antibody of claim 1, wherein the dissociation constant  $K_d$  is 10 equal to or lower than 0.08 nM.

4. The monoclonal antibody of claim 1, wherein the dissociation constant  $K_d$  is equal to or lower than 0.05 nM.

15 5. The monoclonal antibody of claim 1, wherein the dissociation constant  $K_d$  is equal to or lower than 0.01 nM.

6. The monoclonal antibody of claim 1, wherein the dissociation constant  $K_d$  is equal to or lower than 0.005 nM.

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7. The monoclonal antibody of claim 1, wherein the antibody is in a form of scFv.

8. The monoclonal antibody of claim 1, wherein the antibody is in a form of Fab.

25 9. The monoclonal antibody of claim 1, wherein the antibody is in a form of fully assembled antibody.

10. The monoclonal antibody of claim 1, wherein the antibody is in a form of scFv and the dissociation constant  $K_d$  is measured at about 4°C, 25°C, 37°C or 42°C.

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11. The monoclonal antibody of claim 1, wherein the antibody is in a form of Fab and the dissociation constant  $K_d$  is measured at about 4°C, 25°C, 37°C or 42°C.

12. The monoclonal antibody of claim 1, wherein the antibody is in a form of Fab and 5 the dissociation constant  $K_d$  is measured at about 37°C.

14. A monoclonal antibody that specifically binds to a human VEGF and has  $V_L$  comprising the amino acid sequence of

$X_1X_2X_3X_4TQX_5PSX_6X_7SX_8X_9X_{10}GX_{11}X_{12}X_{13}X_{14}IX_{15}CX_{16}X_{17}\underline{SX}_{18}X_{19}IX_{20}X_{21}X_{22}X_{23}X_{24}$

10 WYQQX<sub>25</sub>PGX<sub>26</sub>APX<sub>27</sub>X<sub>28</sub>LX<sub>29</sub>YX<sub>30</sub>X<sub>31</sub>X<sub>32</sub>X<sub>33</sub>LX<sub>34</sub>X<sub>35</sub>GVX<sub>36</sub>X<sub>37</sub>RFSGX<sub>38</sub>X<sub>39</sub>SGTDF X<sub>40</sub>LTI<sub>41</sub>X<sub>42</sub>LQX<sub>43</sub>X<sub>44</sub>DX<sub>45</sub>AX<sub>46</sub>YYCQQX<sub>47</sub>X<sub>48</sub>X<sub>49</sub>X<sub>50</sub>PX<sub>51</sub>TFGX<sub>52</sub>GTKX<sub>53</sub>X<sub>54</sub>IK, wherein the underlined regions are designated as  $V_L/CDR1$ ,  $V_L/CDR2$ , and  $V_L/CDR3$ , respectively, whereas the rest of the region is designated as framework, and wherein  $X_1$  is D, E or A;  $X_2$  is I, or T;  $X_3$  is V, E, K, R, Q, or T;  $X_4$  is M, or L;  $X_5$  is S, or T,  $X_6$  is S, 15 or T;  $X_7$  is L, or V;  $X_8$  is A, or V;  $X_9$  is S, or T;  $X_{10}$  is P, V, L, A, or I;  $X_{11}$  is E, or D;  $X_{12}$  is R, or T;  $X_{13}$  is A, or V I;  $X_{14}$  is T, or A;  $X_{15}$  is T, S, or A;  $X_{16}$  is S, R, N, K, H, or Q;  $X_{17}$  is A, or S;  $X_{18}$  is Q, or R;  $X_{19}$  is S, D, A, or P;  $X_{20}$  is S, G, R, T, or Y;  $X_{21}$  is T, N, S, D, or K;  $X_{22}$  is Y, or D;  $X_{23}$  is L, or I;  $X_{24}$  is A, N, or T;  $X_{25}$  is K, or I;  $X_{26}$  is Q, K, T, or I;  $X_{27}$  is R, K, Q, N, H, S, or E;  $X_{28}$  is V, or L;  $X_{29}$  is I, or V;  $X_{30}$  is F, A, G, D, or S; 20  $X_{31}$  is A, or T;  $X_{32}$  is S, or T;  $X_{33}$  is N, S, R, or T;  $X_{34}$  is A, H, or Q;  $X_{35}$  is S, or G;  $X_{36}$  is P, T;  $X_{37}$  is S, N, D, G, or Y;  $X_{38}$  is S, or T;  $X_{39}$  is G, or R;  $X_{40}$  is T, or A;  $X_{41}$  is S, or R;  $X_{42}$  is S, or R;  $X_{43}$  is P, or A;  $X_{44}$  is E, or D;  $X_{45}$  is F, V, or S;  $X_{46}$  is V, T, I, A, or S;  $X_{47}$  is Y, or S;  $X_{48}$  is S, Y, or N;  $X_{49}$  is S, or T;  $X_{50}$  is T, V, A, P, K, G, S, or I;  $X_{51}$  is W, or Y;  $X_{52}$  is Q, or G;  $X_{53}$  is V, or L; and  $X_{54}$  is E, D, or A.

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15. A monoclonal antibody that specifically binds to a human VEGF and has  $V_L$  comprising the amino acid sequence of

$X_1X_2X_3LTQPPSX_4SX_5TPGQX_6VTISCSGX_7X_8\underline{SNX}_9GX_{10}NX_{11}VX_{12}WYQQX_{13}PGX_{14}A$

PKX<sub>15</sub>LX<sub>16</sub>YX<sub>17</sub>NX<sub>18</sub>X<sub>19</sub>RPSGVPX<sub>20</sub>RX<sub>21</sub>SGSX<sub>22</sub>SX<sub>23</sub>TSASLAISGLX<sub>24</sub>SEDEADYY

30  $CX_{25}X_{26}\underline{WDDSLX}_{27}GYVFGX_{28}GTX_{29}LTVL$ , wherein the underlined regions are designated as  $V_L/CDR1$ ,  $V_L/CDR2$ , and  $V_L/CDR3$ , respectively, whereas the rest of the

region is designated as framework, and wherein X<sub>1</sub> is Q L, or N; X<sub>2</sub> is P A F, or S; X<sub>3</sub> is V, or M; X<sub>4</sub> is A, or T; X<sub>5</sub> is G, or A; X<sub>6</sub> is R, or S; X<sub>7</sub> is S, or T; X<sub>8</sub> is S, T Y, or N; X<sub>9</sub> is I, or V; X<sub>10</sub> is S, or R; X<sub>11</sub> is S, P, N, A, or T; X<sub>12</sub> is N, T , or Y; X<sub>13</sub> is L, or F; X<sub>14</sub> is T, or A; X<sub>15</sub> is V, L, or F; X<sub>16</sub> is M, or I; X<sub>17</sub> is G, T, or S; X<sub>18</sub> is N, or D; X<sub>19</sub> is Q, or E; X<sub>20</sub> is D, or E; X<sub>21</sub> is F, or L; X<sub>22</sub> is K, or R; X<sub>23</sub> is G, or A; X<sub>24</sub> is Q, L, or R; X<sub>25</sub> is A, or G; X<sub>26</sub> is A, S, or T; X<sub>27</sub> is N, S, or T; X<sub>28</sub> is T, or A; and X<sub>29</sub> is K, or Q.

16. A monoclonal antibody that specifically binds to a human VEGF and has V<sub>L</sub> comprising the amino acid sequence of

10 QSALTQPPSVGAPGQRVTISCTGRSSNIGAGHDVHWYQQLPGTAPKLLIYANDQ RPSGVPDFSDSKSGTSASLGISGLRSEDEADYFCATWDDSLHGYVFGTGTKVTV L (SEQ ID No: 54).

17. A monoclonal antibody is provided that specifically binds to a human VEGF and has V<sub>H</sub> comprising the amino acid sequence of

X<sub>1</sub>X<sub>2</sub>QLVX<sub>3</sub>SGGGX<sub>4</sub>VQPGGX<sub>5</sub>LRLX<sub>6</sub>CAX<sub>7</sub>SGX<sub>8</sub>X<sub>9</sub>X<sub>10</sub>X<sub>11</sub>X<sub>12</sub>X<sub>13</sub>GX<sub>14</sub>NWX<sub>15</sub>RQAP  
GKGX<sub>16</sub>EWVGWX<sub>17</sub>NTX<sub>18</sub>X<sub>19</sub>GX<sub>20</sub>X<sub>21</sub>TYX<sub>22</sub>X<sub>23</sub>X<sub>24</sub>FX<sub>25</sub>RRX<sub>26</sub>TX<sub>27</sub>SX<sub>28</sub>X<sub>29</sub>X<sub>30</sub>SKX<sub>31</sub>  
X<sub>32</sub>X<sub>33</sub>YLQX<sub>34</sub>NSLRAEDTAVYYCAX<sub>35</sub>YPX<sub>36</sub>YYGX<sub>37</sub>SHWYFDVWX<sub>38</sub>QGTLVTV SS, wherein the underlined regions are designated as CDR1, CDR2, and CDR3,

20 respectively, whereas the rest of the region is designated as framework according to Kabat nomenclature, and wherein X<sub>1</sub> is E, or Q; X<sub>2</sub> is V, or G; X<sub>3</sub> is Q, or E; X<sub>4</sub> is V, or L; X<sub>5</sub> is S, or T; X<sub>6</sub> is S T, or R; X<sub>7</sub> is A, or V; X<sub>8</sub> is Y, or F; X<sub>9</sub> is T, D, N, S, or A; X<sub>10</sub> is F, or L; X<sub>11</sub> is T, D, Y, A, S, or N; X<sub>12</sub> is N, H, or S; X<sub>13</sub> is Y, or F; X<sub>14</sub> is M, L, I, or V; X<sub>15</sub> is I, V, or L; X<sub>16</sub> is L, or P; X<sub>17</sub> is I, or V; X<sub>18</sub> is Y, or N; X<sub>19</sub> is T, or N; X<sub>20</sub> is E, or A; X<sub>21</sub> is P, T, or S; X<sub>22</sub> is A, or V; X<sub>23</sub> is A, H, Q, P, D, or E; X<sub>24</sub> is D, or E; X<sub>25</sub> is K, or T; X<sub>26</sub> is V, F, or L; X<sub>27</sub> is F, or I; X<sub>28</sub> is L, or R; X<sub>29</sub> is D, or N; X<sub>30</sub> is T, or N; X<sub>31</sub> is S, or N; X<sub>32</sub> is T, Q, P, or K; X<sub>33</sub> is A, V, or P; X<sub>34</sub> is L, or M; X<sub>35</sub> is K, or R; X<sub>36</sub> is H, or Y; X<sub>37</sub> is S, R, or T; and X<sub>38</sub> is G, or A.

30 18. A monoclonal antibody is provided that specifically binds to a human VEGF and has V<sub>L</sub> comprising the amino acid sequence selected from the group consisting of SEQ ID

- NOs:2-54, more preferably comprising the amino acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:44, SEQ ID NO:47, and SEQ ID NO:54.
- 5 19. A monoclonal antibody that specifically binds to a human VEGF and has  $V_H$  comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:57-110 and SEQ ID NOs:285-310, and preferably comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:61-64, SEQ ID NO:67, 68, 70, 75, 83, 88, 89, 90, 91, 92, 93, 94, and 96-110.  
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- 20. A monoclonal antibody is provided that specifically binds to a human VEGF and has CDR2 in the  $V_L$  region ( $V_L/CDR2$ ) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:195-209.
- 15 21. A monoclonal antibody that specifically binds to a human VEGF and has CDR3 in the  $V_L$  region ( $V_L/CDR3$ ) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:210-228.
- 22. A monoclonal antibody that specifically binds to a human VEGF and has a framework region (FR) CDR3 in the  $V_L$  region ( $V_L/FR$ ) comprising the amino acid sequence selected from the group consisting of: SEQ ID NO:229-269, and preferably comprising the amino acid sequence selected from the group consisting of SEQ ID NO:232, 235, 237, 251, 255, 263, and 265.  
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- 25 23. A monoclonal antibody that specifically binds to a human VEGF and has CDR1 in the  $V_H$  region ( $V_H/CDR1$ ) comprising the amino acid sequence of  $GX_1X_2X_3X_4X_5X_6GX_7N$ , wherein  $X_1$  is Y, or F;  $X_2$  is D, N, T, S, or A;  $X_3$  is F, or L;  $X_4$  is T, D, S, Y, A, or N;  $X_5$  is H, N, or S;  $X_6$  is Y, or F;  $X_7$  is M, L, I, or V.
- 30 24. A monoclonal antibody that specifically binds to a human VEGF and has CDR2 in the  $V_H$  region ( $V_H/CDR2$ ) comprising the amino acid sequence of

WX<sub>1</sub>NTX<sub>2</sub>X<sub>3</sub>GEX<sub>4</sub>TYX<sub>5</sub>X<sub>6</sub>X<sub>7</sub>FX<sub>8</sub>R, wherein X<sub>1</sub> is I, or V; X<sub>2</sub> is Y, or N; X<sub>3</sub> is T, or N; X<sub>4</sub> is P, T, or S; X<sub>5</sub> is A, or V; X<sub>6</sub> is A, Q, P, H, D, or E; X<sub>7</sub> is D, or E; and X<sub>8</sub> is K, or T.

25. A monoclonal antibody that specifically binds to a human VEGF and has CDR2 in the V<sub>H</sub> region (V<sub>H</sub>/CDR2) comprising the amino acid sequence selected from the group consisting of: SEQ ID NOs:136-156.

26. A monoclonal antibody that specifically binds to a human VEGF and has CDR3 in the V<sub>H</sub> region (V<sub>H</sub>/CDR3) comprising the amino acid sequence of KYPX<sub>1</sub>YYGX<sub>2</sub>SHWYFDV, wherein X<sub>1</sub> is Y, or H, and X<sub>2</sub> is R.

27. A monoclonal antibody that specifically binds to a human VEGF and has CDR3 in the V<sub>H</sub> region (V<sub>H</sub>/CDR3) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs:311-337.

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28. A monoclonal antibody that specifically binds to a human VEGF and has FR in the V<sub>H</sub> region (V<sub>H</sub>/FR) comprising the amino acid sequence of X<sub>1</sub>VQLVX<sub>2</sub>SGGGX<sub>3</sub>VQPGGX<sub>4</sub>LRLX<sub>5</sub>CAX<sub>6</sub>S/CDR1/WX<sub>7</sub>RQAPGKGLEWVG/CDR2//RX<sub>8</sub>TX<sub>9</sub>SX<sub>10</sub>DX<sub>11</sub>SKX<sub>12</sub>X<sub>13</sub>X<sub>14</sub>YLQX<sub>15</sub>NSLRAEDTAVYYCA/CDR3/WX<sub>16</sub>QGTLVTV

20 SS, wherein X<sub>1</sub> is E, or Q; X<sub>2</sub> is Q, or E; X<sub>3</sub> is V, or L; X<sub>4</sub> is S, or T; X<sub>5</sub> is S, T, or R; X<sub>6</sub> is A, or V; X<sub>7</sub> is I, or V; X<sub>8</sub> is F, or V; X<sub>9</sub> is F, or I; X<sub>10</sub> is L, or R is X<sub>11</sub> is T, or N; X<sub>12</sub> is S, or N; X<sub>13</sub> is T, Q, or K; X<sub>14</sub> is A, or V; X<sub>15</sub> is M, or L; and X<sub>16</sub> is G, or A.

29. A monoclonal antibody that specifically binds to a human VEGF and has a V<sub>L</sub> and V<sub>H</sub> pair selected from the group consisting of: SEQ ID NO:1 and 70; SEQ ID NO:1 and 67; SEQ ID NO:1 and 75; SEQ ID NO:1 and 83; SEQ ID NO:14 and 55; SEQ ID NO:1 and 101; SEQ ID NO:1 and 100; SEQ ID NO:14 and 102; SEQ ID NO:1 and 103; SEQ ID NO:1 and 104; SEQ ID NO:1 and 105; SEQ ID NO:36 and 100; SEQ ID NO:26 and 100; SEQ ID NO:28 and 100; SEQ ID NO:37 and 100; SEQ ID NO:44 and 100;

30 SEQ ID NO:54 and 100; and SEQ ID NO:47 and 100, preferably selected from the group consisting of SEQ ID NO:28 and 61; SEQ ID NO:28 and 62; SEQ ID NO:28 and 63;

SEQ ID NO:28 and 64; SEQ ID NO:28 and 68; SEQ ID NO:28 and 85; SEQ ID NO:28 and 86; SEQ ID NO:28 and 87; SEQ ID NO:28 and 88; SEQ ID NO:28 and 89; SEQ ID NO:28 and 90; SEQ ID NO:28 and 91; SEQ ID NO:28 and 92; SEQ ID NO:28 and 93; SEQ ID NO:28 and 94; SEQ ID NO:28 and 95; SEQ ID NO:28 and 96; SEQ ID NO:28 and 97; SEQ ID NO:28 and 98; SEQ ID NO:28 and 99; SEQ ID NO:28 and 106; SEQ ID NO:28 and 107; SEQ ID NO:28 and 108; SEQ ID NO:28 and 109; and SEQ ID NO:28 and 110.

5       30.     The monoclonal antibody of any of claims 14-29, wherein the antibody has  
10      dissociation constant  $K_d$  equal to or lower than 10 nM.

31.     The monoclonal antibody of any of claims 14-29, wherein the antibody has  
dissociation constant  $K_d$  equal to or lower than 1 nM.

15    32.     The monoclonal antibody of any of claims 14-29, wherein the antibody has  
dissociation constant  $K_d$  equal to or lower than 0.1 nM.

33.     The monoclonal antibody of any of claims 14-29, wherein the antibody has  
dissociation constant  $K_d$  equal to or lower than 0.01 nM.